0508-1044

IN THE U.S. PATENT AND TRADEMARK OFFICE

In re application of

Jacques Alexandre HATZFELD et al. Conf. 5595

Application No. 09/980,484 Group 1632

Filed March 25, 2002 Examiner Thaian N. Ton

PROCESS FOR THE MULTIPLICATION OF

STEM CELLS

DECLARATION UNDER RULE 132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Jacques Alexandre HATZFELD, hereby declare as follows:

My relevant background and experience are set forth on the attached c.v. I make this declaration in support of the present application, and to provide evidence in rebuttal of several contentions set forth in the outstanding Official Action.

In particular, I make this declaration to rebut the contention that one skilled in the art would not be able to practice the claimed invention with activin or with stem cells other than hematopoietic stem cells.

I declare that the present disclosure plainly teaches the steps and conditions required so that one skilled in the art can practice the claimed invention with activin and stem cells other than hematopoietic stem cells. In support of my position, I submit the results of experiments that utilize activin and embryonic stem cells in accordance with the teachings of the specification. The results are as follows:

I. Effect of TGFß and Activin on Human Embryonic Stem Cells (hESCs)
Cultured in a Synthetic Serum-Free Defined Medium

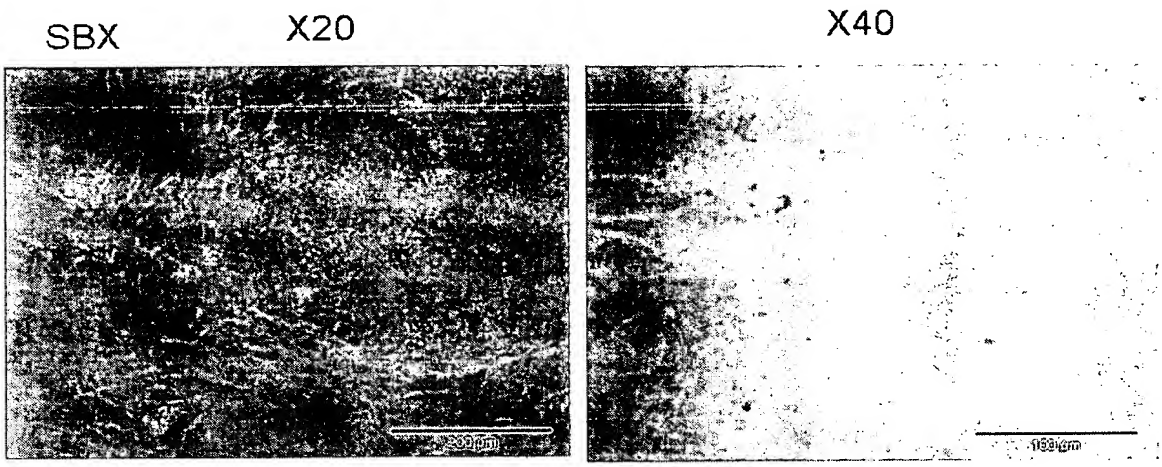
HESCs were cultured on human matrices in a liquid synthetic serum-free medium called SBX. Figures 1A, B, C and D show pictures taken at 200x and 400x enlargement, showing clearly the differences between control (without TGFβ) and TGFß supplemented wells.

When added to SBX, TGFβ (500 pg/ml) allows the stem cells to maintain their primitive state. In other words, the cells remain a small size and maintain tight junctions between the cells. A dramatic difference is observed with the cells grown in SBX medium without TGFβ or activin. These cells undergo differentiation. In particular, the cells become larger and lose their tight junctions.

Figure 2 shows the percentage of cells that divide while maintaining the undifferentiated state, which is reflected by the percentage of SSEA3 expression, a marker of embryonic stem cell primitivity.

Noggin (100 ng/ml) is used as the anti-TGFβ compound. The cells that are treated with 500 pg/ml TGFß and/or 30 ng/ml activin maintain primitivity. Moreover, the use of noggin in addition to activin further enhances the primitivity of the cells.

Figure 1



SBX + TGFbeta 500pg/ml

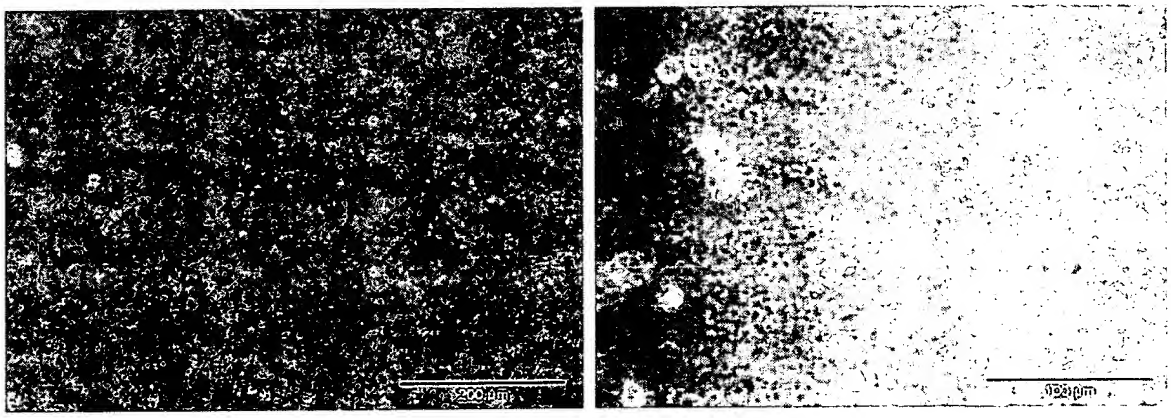
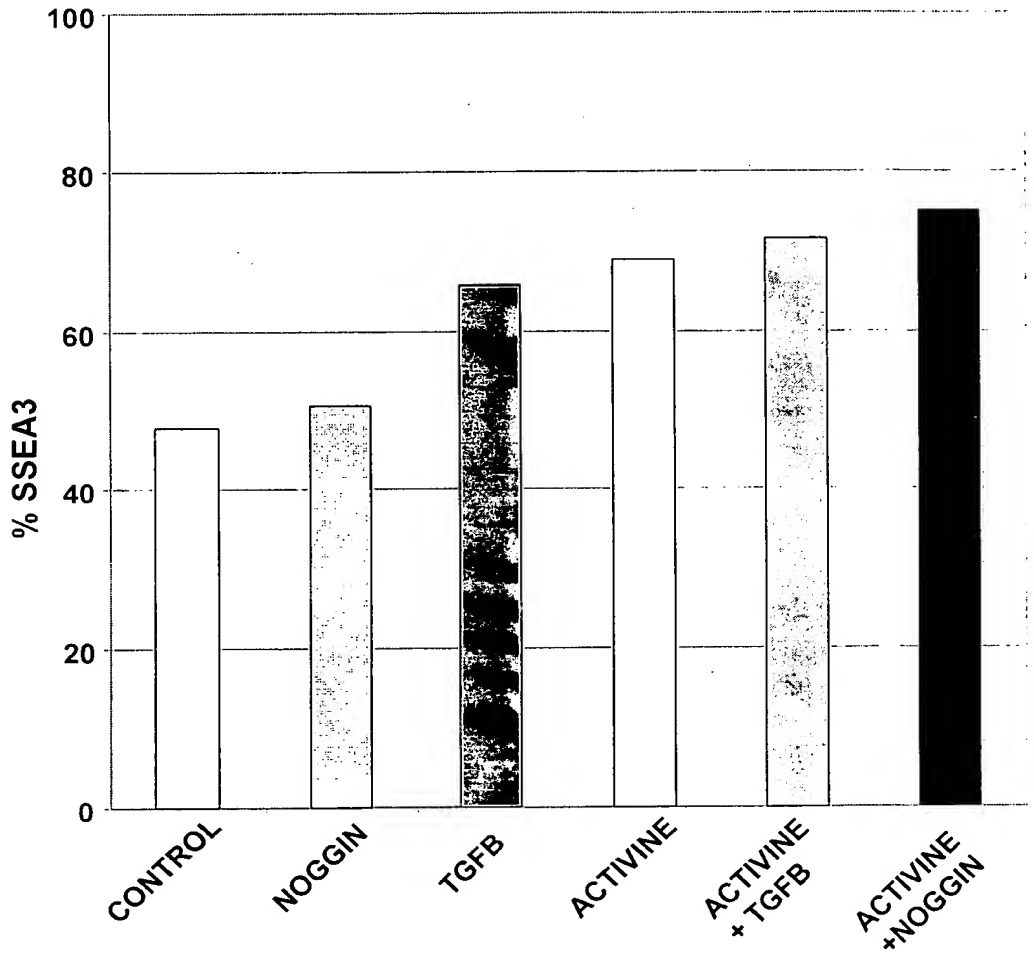


Figure 2


100
80
60
40
20
0
% SSEA3
CONTROL
NOGGIN
TGFB
ACTIVINE
ACTIVINE + TGFB
ACTIVINE +NOGGIN

II. Effect of Activin on Human Embryonic Stem Cells (hES2)

The differentiation gene expression pattern of hES2 was also assessed in the presence or absence of Activin A (30ng/ml) and Noggin (100ng/ml).

Figure 3

		hES2 on MEF SR+FGF2	SBX +Activin 30ng/ml	SBX +Activin 30ng/ml +Noggin 100ng/ml
AMPLIFICATION (Fold)		6	8	6
MESODERM	T	-	+	-
	ENG	-	+	-
ECTODERM	NEUROG1	-	+	-
	SOX17	-	+	-
ENDODERM	FOXA2	-	+	-
	GATA6	-	+	-
	GATA4	-	+	-

"hES2 on MEF in SR (Serum Replacement) + FGF2" corresponds to standard culture conditions : hES2 cells are cultivated on a mouse embryonic fibroblast layer in a medium SR supplemented with FGF2.

" + " means gene expression and " - " means no gene expression.

T and ENG are markers of stem cell differentiation of the mesoderm. NEUROG1 and SOX17 are markers of stem cell differentiation of the ectoderm. FOXA2, GATA6 and GATA4 are markers of stem cell differentiation of the endoderm.

Human embryonic stem cells in presence of activin (30 ng/ml) divide faster than the in the control conditions or than in the presence of activin and noggin, but express all the tested differentiation markers. Thus, self-renewal is not achieved when activin is used alone.

This stands in contrast to the results obtained when noggin and activin are added in the SBX medium. Indeed, the addition of the anti-inhibitor of cell proliferation (noggin), allows the cells to divide while maintaining their undifferentiated state.

Thus, in view of the above, it is believed to be apparent that the claimed invention can be practiced with stem cells other than hematopoietic stem cells (e.g., embryonic stem cells) and activin by following the teachings of the claimed invention.

The undersigned declares further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jacques Alexandre Hatzfeld December 12, 2006

CURRICULUM VITAE

HATZFELD Jacques, Ph.D.,
CNRS Research Director First Class.
Head of the CNRS Human Stem Cell Laboratory,
CNRS
7, rue Guy Moquet
94800- VILLEJUIF, FRANCE.

Nationality : French

Marital status : Married, two children.

University Titles :

1966 Ingenieur Agronome Specialty: Plant Genetics at Institut National Agronomique, Paris.
Maîtrise (M.S.): Biochemistry-Genetics-General Biology
1967 DEA Microbiology, Orsay, Science University
1972 Ph.D., Paris VI Sciences University.

Research Carrier :

1967 Fellow from Anticancer National League
1969 CNRS Probationer
1970 CNRS Research Attaché
1975 CNRS Research Chargé
1986 CNRS Research Director II
1996-1998 : Chief of the CNRS Unit "Cell Engeneering"
1999 CNRS Research Director I

Current Responsability :

Head of the C.N.R.S Human Stem Cell Laboratory, Institut Andre Lwoff, Villejuif

WORKING PLACE- MAIN RESEARCH SUBJECTS

1966-1972 : Radium Institute, Orsay, France.
SUBJECT : DNA Repair and Cell Cycle

1972-1977 : Molecular Biology Research Institute, Genetic Department,Paris, France.
SUBJECT : Cell Cycle Genetic.

1977-1980 : The Rockefeller University, New-York, U.S.A., Laboratory of Biological Chemistry.
SUBJECT : Receptor Expression.

1980-1984 : Cellular Pathology Institute, Kremlin-Bicêtre, France
SUBJECT : Human Bone Marrow Stem Cell Assay in Serum-free Medium.

1985-1992 : Institut de Cancérologie et d'Immunogénétique, Villejuif, France. Head of the Laboratory of Cell and Molecular Biology of cytokines.
SUBJECT : Receptor and Gene Expression Induced by Human Bone Marrow Stem Cell Growth Factors.

1993-1998 CNRS : Institut de Recherche Scientifique sur le Cancer (IRSC), Villejuif, France.
SUBJECT : Human Hematopoietic Stem Cell. Cell cycle and differentiation controls.

1998-2002 CNRS : Institut de Recherche Scientifique sur le Cancer (IRSC), Villejuif, France.
SUBJECT : Human Stem Cell. Cell Cycle and Differentiation Controls.

2002-2006 CNRS Human Stem Cell Laboratory Institut André Lwoff Villejuif
SUBJECT : Human Embryonic and Adult Stem Cells

PATENTS

1984 Serum-free Medium for Hybridomas, INPI n° 8400177.

1994 Method for gene transfert into cells activated from a quiescent state, INPI n° 94.15497. US Patent # 08/860,299

1999 Divisional Patent of the former Patent (n° 94 15497)

1999 Registration of a Patent " Process for the multiplication of Stem Cells".

2002 Enrichment technique of keratinocyte stem cells.

2006 Primitive endodermal stem cells, a process for preparing them and their use, in particular for obtaining primitive epithelial liver cells

EUROPEAN CONTRACTS

European Concerted Action

1990-1993: Project Leader of the "Human Bone Marrow Stem Cell" European Concerted Action.

1994-1996: Member of the Management Board of the "Human Hematopoietic Stem Cell" European Concerted Action.

European Contract Biotechnology

1997-2000: Coordinator of the Biotech contract "Bioreactor Production of Human Haematopoietic Cells"

WORKSHOP ORGANIZER

1985 Paris. Cell Culture in Serum-free Media.

1989 Villejuif, FRANCE, Commission of the European Commities (CEC): First Workshop "Human Bone Marrow Stem Cell"

1990 Rijswijk, NEDERLANDS, CEC- Second Workshop "Human Bone Marrow Stem Cell"

1991 Villejuif, FRANCE, Third Workshop "Human Bone Marrow Stem Cell"

1991 Villejuif, FRANCE, 1st Workshop on CD34+ Cells and Bone Marrow Transplantation

1992 Paris, FRANCE, Fourth Workshop "Human Bone Marrow Stem Cell"

1993 Villejuif, FRANCE, Workshop : Comparison of CD34+ Cell Separation Devices.

1994 Brussel, BELGIUM, First Meeting of the European Haematology Association. Worshop of the European Concerted Action on Human Haematopoietic Stem Cells.

1995 Düsseldorf, GERMANY, 24th Meeting of the International Society of Experimental Hematology (ISEH), Workshop : "Novel receptors on $CD34^+$ cells and the function of CD34".

1996 Paris, FRANCE, 2d Meeting of the European Haematology Association, Workshop of the European Concerted Action on Human Stem Cells : "From theory to cell therapy".

VISITING SCIENTIST

1971	National Institute for Medical Research, Mill Hill, London, NW7. U. K. (1 month) Dr. D. H. Williamson
1976	Massachusetts Institute of Technology, Cancer Center, Cambridge, Mass., U.S.A. (2 months) Dr. W.G. Thilly
1977-1979	The Rockefeller University, New-York, N.Y., U.S.A. (22 months) Pr. E. Reich
1979-1980	The Rockefeller University, New-York, N.Y., U.S.A. (13 months) Pr. H. G. Kunkel
1980	Department of Biochemistry, Q 058, University of California, San Diego, La Jolla, U.S.A. (1 month) Pr. G. H. Sato
1980	Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, U.K. (1 month) Dr. T.M. Dexter & Pr. L. G. Lajtha
1981	Ontario Cancer Institute, Toronto, Ontario,Canada. (1 month) Dr. H. A. Messner & Dr. E.A. McCulloch
1984	Anderson Hospital, University of Texas, U.S.A. (1 month) Dr. A. Maizel
	Veterans Administration Medical Center, Charleston, U.S.A. Dr. M. Ogawa
1989	Leukemia Research Fund Center at the Institute of Cancer Research, Chester Beatty Laboratories, London, United Kingdom Dr M. F. Greaves
1990	Genetics Institute, Cambridge, U.S.A. Dr S. Clark
1991	Indiana University, Department of Medicine, Hematology/Oncology Section Dr R. Hoffman & Dr E. Srour
1991	New York Blood Center Drs G. & A. R. Migliaccio
1991	Harvard Medical School, Beth Hospital Dr B. Lim

1991 Applied Immune Sciences, Santa Clara CA ,
Drs T. Okarma, J Lebkowski.

1993 Indiana University, Department of Medicine, Hematology/Oncology Section Dr. E. Srour.

1994 Cambridge, U.K. National Blood Transfusion Service.

1995 Expert for ECVAM, Milano, Italy

2000 Expert for the European Space Agency, Zürich, Switzerland

2001 and 2002 : Visit to Monash Institute and ES Cell International in Melbourne Australia

2002 Visit to Hubrecht Laboratory Utrecht. NL

2003 Genome Institute of Singapore SG

2005 Kyoto University Japan

2006 Kunming Medical University, China

EMERITUS PROFESSOR

Emeritus Professor of the University of Kunming, China